functions of Syt1. However, in a number of assays Syt1 and C2AB behave differently, indicating that C2AB may not fully mimic the activity of the full-length protein. Detailed conformational studies of full-length Syt1 have not been reported and in the present work we employ EPR spectroscopy to investigate the state of the linker that attaches the C2A and C2B domains to the vesicle membrane in the full-length protein. CW-EPR spectra and double electron-electron resonance (DEER) distance measurements of single spin-labeled Syt1 indicate that the juxta-membrane linker remains closely associated with the membrane interface and acts to oligomerize full-length Syt1 in the absence of calcium. EPR data also demonstrate that a membrane associated glycine zipper/GXXXG motif in juxta-membrane linker is playing a crucial role in this intermolecular association. Using a total internal reflection fluorescence (TIRF) assay we measure the ability of Syt1 to capture liposomes that mimic the target plasma membrane. The TIRF binding assay shows that the ability of Syt1 to oligomerize through this linker plays a role in the ability of syt1 to interact with target membranes. The membrane binding activity of Syt1 likely plays a key role in triggering membrane fusion. Our detailed structural information provides a basis for understanding the different Ca^{2+}-dependent activities of the full-length Syt1 and the soluble C2AB construct in in-vitro fusion assays that involve isolated reconstituted components of the fusion system.

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Measuring the Impact of Lipid Interactions on the Mobility and Localization of Synaptic Proteins in Live Synapses

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The presynaptic protein complexin can both promote and inhibit fusion through interactions between its central helix and the SNARE complex. A poorly conserved C-terminal domain (CTD) is also required for inhibition of spontaneous fusion. We found that the CTD binds lipids through a novel protein motif and directs complexin onto synaptic vesicles where it can efficiently engage the SNAREs and inhibit spontaneous fusion. Using in vivo dynamic imaging approaches in C. elegans, we observed that complexin is sequestered within presynaptic terminals through its CTD while its escape rate out of the synapse depends sensitively on synaptic activity. Complexin exhibits reduced mobility in synaptic boutons compared to neighboring axonal regions and its mobility is enhanced when synaptic vesicles were removed, consistent with their role in capture and retention of complexin. Finally, several common lipid-interacting protein motifs were imaged at synapses in the presence and absence of synaptic activity, and the impact of disrupting these lipid-binding domains was quantified. Simple one-dimensional reaction diffusion models were used to quantify the dynamics of protein exchange between en passant synapses.

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Guided Growth of Neurons on Micro-Structured Surfaces

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Synaptic cell adhesion molecules (SCAMs) are well known to interact across the synaptic cleft of central mammalian synapses. However, their functional role in transsynaptic modulation of the synaptic vesicle cycle (i.e. from the postsynaptic to the presynaptic neuron) is poorly understood. Several families of SCAMs have been characterized at the molecular level. Transsynaptic interactions mediated by SCAMs potentially control initial synapse formation, regulate structural maturation of synapses, modulate basal synaptic function including vesicle endocytosis, and participate in different forms of long-term synaptic plasticity.

In order to better separate pre- and postsynaptic effects we grew neurons on microstructured surfaces, functionalized with SCAM proteins. Glass coverslips were patterned with the Neurexin-binding Neuroligin fragment via microcontactprinting by either coating them with silanes to which we covalently coupled Neuroligin with cysteine tag, or with Poly-l-lysine-polyethylenglycol-HaloTag-ligand, covalently linked to Neuroligin via a HaloTag.

Both approaches lead to controlled and guided growth of neuronal outgrowths. Formation of presynaptic sites was triggered within one to two days. These sites showed a positive staining with antibodies against the active zone markers RIM1/2 and the synaptic vesicle protein Synaptophysin1. They often opposed the structured Neuroligin patches, as revealed by 4Pi microscopy. Such varicosities contained vesicles that could be stained with FM 1-43 upon electrical stimulation. Release of FM 1-43 by repeated stimulation could be monitored by TIRF microscopy, displaying similar kinetics as control synapses.

Formation of synapses on structured surfaces opens up the possibility to study presynapse formation and dynamics under controlled conditions.

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Cerebellar Interneurons use Dendritic Voltage and Calcium Signals to Differentially Extract Information from Synaptic Activity

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Non-linear summation of synaptic inputs within a dendritic branch endows neurons with multiple computational subunits, favoring different types of pattern detection. To date, most neuronal types have been described to display a supralinear summation of synaptic inputs, due to the activation of NMDAR or voltage-gated calcium channels, with a concomitant supralinearity of local Ca^{2+} signaling. Recently, evidence showed that in dendrites of cerebellar stellate cells, synaptic summation is sublinear, likely resulting from a reduced driving-force for synaptic currents caused by large local depolarizations. We expect these large synaptic depolarizations to cause a smaller fractional change in the driving force for Ca^{2+}, therefore a more linear summation of dendritic Ca^{2+}.

In order to characterize the local Ca^{2+} and voltage responses to synaptic stimulation in cerebellar stellate cells, we combined two-photon targeted stimulation of parallel fibers, glutamate uncaging, and fast two-photon imaging of dendritic Ca^{2+} and voltage. Using fast line-scan imaging and the two-component voltage sensor DiO/DPA , we observed that, in accordance with numerical simulations, the local depolarization in the dendrites of stellate cells in response to the activation of a few synapses is rapid (<2ms), of large amplitude (up to 50mV) and distance-dependent, and is widely spread in the dendrite (several tens of µms). The measurement of Ca^{2+} transients in stellate cells dendrites showed that, in contrast, Ca^{2+} transients are more localized (<10µm), summed linearly in response to paired stimulation of parallel fibers, and supra-linearly in response to synaptic activation by high-frequency trains.

Therefore Ca^{2+} and voltage in dendrites can obey different computational rules, the sublinear summation of voltage contributing to sparse-input detection and shaping the activation of Purkinje cells by parallel fibers, while supralinear Ca^{2+} is likely to contribute to regulation of synaptic plasticity.

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Defects in Synapse Structure and Function in a Fly Model of FUS-Related ALS

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Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that leads invariably to fatal paralysis. Although most cases of ALS are sporadic, about 10% are familial. One gene associated with familial ALS encodes the DNA/RNA binding protein Fused in Sarcoma (FUS). There exists a Drosophila model of ALS, in which human FUS with ALS-causing mutations is expressed in motor neurons. These flies exhibit motor neuron degeneration, larval locomotor defects and early death. Similar phenotypes are observed in flies null for the gene Cabeza (Caz), the fly homolog of FUS. We have examined evoked and spontaneous synaptic transmission at the larval neuromuscular junction, larval motor neuron cell body excitability, and presynaptic active zone structure in these fly models of ALS. The amplitude of evoked synaptic currents is decreased by more than 80% in larvae in which human mutant FUS (R521C) is expressed in motor neurons. A similar decrease in evoked synaptic transmission is seen in Caz1 null flies. Furthermore, the frequency of spontaneous miniature synaptic currents is decreased dramatically in FUS-R521C expressing flies. In marked contrast, recordings from motor neuron cell bodies demonstrate that both wild type and mutant FUS expressing neurons can fire normal action potentials, and the voltage-dependent inward and outward currents in the cell bodies are indistinguishable in wild type and mutant FUS motor neurons. Although confocal microscopic analysis of the larval neuromuscular junction does not reveal gross abnormalities, examination of synapses using super-resolution STED microscopy suggests that presynaptic active zones are aberrantly organized in larvae in which FUS-R521C is expressed in the motor neurons. The results are consistent with the idea that